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**Toxic Effects of Cadmium on Three
Generations of Brook Trout
(*Salvelinus fontinalis*)**

Environmental Research Lab -Duluth, Minn

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Toxic Effects of Cadmium on Three Generations of Brook Trout (*Salvelinus fontinalis*)

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ABSTRACT

Three generations of brook trout (*Salvelinus fontinalis*) were exposed to several concentrations of total cadmium (0.06-6.4 $\mu\text{g Cd/liter}$). Significant numbers of first- and second-generation adult males died during spawning at 3.4 $\mu\text{g Cd/liter}$. This concentration also significantly retarded growth of juvenile second- and third-generation offspring. The maximum acceptable toxicant concentration (MATC) for brook trout exposed to cadmium in Lake Superior water (hardness 44 mg/liter as CaCO_3 , pH 7-8) lies between 1.7 and 3.4 $\mu\text{g Cd/liter}$. Cadmium-residue analyses of kidney, liver, gill, gonad, spleen, muscle, and red blood cells from first- and second-generation trout indicated that kidney, liver, and gill tissue accumulated the greatest amounts of cadmium at each water exposure concentration. No significant increases in cadmium were measured in edible muscle at any of the cadmium water concentrations tested. Cadmium residues in kidney, liver, and gill tissue of fish from all exposure concentrations reached equilibrium ($\mu\text{g Cd/g tissue}$) in both first- and second-generation trout after 20 weeks. Cadmium loss from gill tissue of second-generation trout placed in control water for 12 weeks was rapid; however, no loss was detectable from the liver and kidney.

Cadmium contamination in the environment and its highly toxic effects on animal life are well documented in several reviews (McKee and Wolf 1963; Nilsson 1970; Flick et al. 1971; Fassett 1975). However, information on long-term or chronic effects of cadmium on survival, growth, and reproduction of fish is rather limited. Pickering and Gast (1972) and Eaton (1974) conducted acute and chronic hard water (200 mg/liter as CaCO_3) cadmium exposures of fathead minnows (*Pimephales promelas*) and bluegills (*Lepomis macrochirus*) to determine the maximum acceptable toxicant concentration (MATC) and subsequent application factors MATC/(96-h LC50) (Mount and Stephan 1967). Results from these tests indicated that the MATC values were 37-57 and 31-80 $\mu\text{g Cd/liter}$ for the fathead minnow and bluegill, respectively. The application factor derived in hard water was 0.005-0.008 for the fathead minnow and 0.002-0.004 for the bluegill.

Our study involved three generations of brook trout (*Salvelinus fontinalis*) exposed to low water concentrations of cadmium over a 3-year period in soft water. It was designed to establish a MATC value based on survival, growth, and reproduction and was also designed to determine the bioaccumulation and loss of cadmium.

METHODS

Physical and Chemical

Exposure System. The exposure system consisted of a proportional diluter (Mount and Brungs 1967) that delivered 2 liters of water per cycle to flow-splitting chambers (Benoit and Puglisi 1973). These chambers divided each of six concentrations four ways into duplicate spawning tanks and duplicate larvae growth tanks. Flow-rates averaged 625 ml/min for spawning tanks and 94 ml/min for larvae growth tanks. Replacement time for 90% of the test water was calculated to be approximately 4.5 h in spawning and larvae growth tanks (Sprague 1969). Spawning tanks were 91 × 31 × 38-cm stainless steel tanks with a 31-cm standpipe. Larvae growth tanks were located directly above and drained into the spawning tanks containing test water of the same concentration. They consisted of an outer glass tank, 55 × 28 × 15 cm, with an inside tank, 43 × 26 × 15 cm, divided lengthwise into two 13-cm wide chambers. Water depth in each chamber measured 13 cm. All tanks were aerated, siphoned Monday through Friday, and scrubbed once a week. Spawning tanks were covered with fiberglass screens and concealed by curtains to minimize outside disturbance. Embryos and alevins up

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TABLE 1.—Survival, spawning, and embryo production of first- and second-generation brook trout exposed to various concentrations of cadmium.

Item	Total cadmium ($\mu\text{g/liter}$)											
											Control	
	6.3 ^a $\pm$ 0.25	6.4 $\pm$ 0.18	3.4 $\pm$ 0.12	3.4 $\pm$ 0.10	1.7 $\pm$ 0.07	1.6 $\pm$ 0.07	0.9 $\pm$ 0.05	0.8 $\pm$ 0.07	0.5 $\pm$ 0.04	0.5 $\pm$ 0.03	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01
	A ^b	B	A	B	A	B	A	B	A	B	A	B
First generation												
Adult survival during spawning (%) (September 1971–February 1972)	0 ^c	0 ^c	40	60	100	100	100	100	100	100	100	80 ^d
Number of mature males	2 ^e	2 ^e	2	2	2	2	2	2	2	2	2	2
Number of mature females	3	3	3	3	3	3	3	3	3	3	3	3
Number of females spawning	0	2	3	1	3	3	2.5	2	3	3	3	3
Mean spawnings per female		1	3	1	3	3	2.7	4.4	3.5	3.0	4.3	3.7
Mean viable embryos spawned per female			136	425	302	709	560	300	378	800	602	
Second generation												
Adult survival during spawning (%) (September 1973–February 1974)	83 ^f	83	100	50 ^d	100	100	*	*	100	100		
Number of mature males	1 ^e	1 ^e	3	2	3	2			2	2		
Number of mature females	5	5	3	1	3	4			4	4		
Number of females spawning	0	1	3	1	3	1			2.5	0.5		
Mean spawnings per female		1.0	3.0	2.0	1.3	2.3			3.0	1.0		
Mean viable embryos spawned per female			269	360	388	193	432				609	446

^a Mean and 95% confidence interval.^b A and B represent duplicate spawning tanks.^c All fish died prior to reaching sexual maturity.^d Disease-related death.^e All males died either during or shortly after spawning.^f No successful reproduction occurred in first-generation trout; therefore, second-generation spawners from B were divided equally between duplicates at time of spawning.^{*} No second-generation fish exposed at this concentration.

to 4 weeks old were protected from direct light by sheets of black plastic and incubated in nearly complete darkness (0.02 lumens). Sylvania Gro-Lux and Duro-Test Vita-Lite fluorescent lamps^{1,2} were used in combination as the main source of illumination. The fluorescent lamps were used in conjunction with incandescent bulbs wired to a light timer that controlled the photoperiod and an automatic dimmer which simulated sunrise and sunset (Drummond and Dawson 1970). Maximum light intensity measured 8.5 lumens at the water surface of the spawning tanks.

Water Characteristics. Water was obtained directly from Lake Superior. It was passed through an ultraviolet sterilizer to kill potential pathogens and was heated to the test temperatures by a hot water heat-exchange system. Seasonally adjusted temperatures ranged from 9 to 15°C throughout the juvenile-adult exposures. Temperatures were main-

tained at $9 \pm 1^\circ\text{C}$ during spawning, embryo incubation, and alevin-juvenile growth studies. Total hardness, alkalinity, acidity, and pH determinations were made on water from all tanks, alternately, one tank per week; dissolved oxygen was measured in a different duplicate tank twice a week. The means and ranges for total hardness, alkalinity, and acidity determinations were 44 (42–47), 43 (38–46), and 4 (1–10) mg/liter as CaCO_3 , respectively. The pH ranged from 7 to 8, and the mean for dissolved oxygen was 7 mg/liter (range 4–12). Chemical measurements were made according to the American Public Health Association et al. (1971).

Toxicant Solution. Cadmium chloride (anhydrous CdCl_2 , reagent grade) was dissolved in distilled water and introduced to the diluter by a chemical-metering device (Mount and Warner 1965). Total cadmium concentrations (Table 1) were assigned by stratified random assignment to each test tank.

Water samples (25 ml/day) were taken Monday through Friday from each spawning and larvae growth tank, acidified to pH 2.5–3.0, and composited for 2-week periods.

¹ The U.S. Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used only for identification.

² Sylvania Lighting Center, Danvers, Massachusetts 01923; and, Duro-Test Corporation, Secaucus, New Jersey 07094.

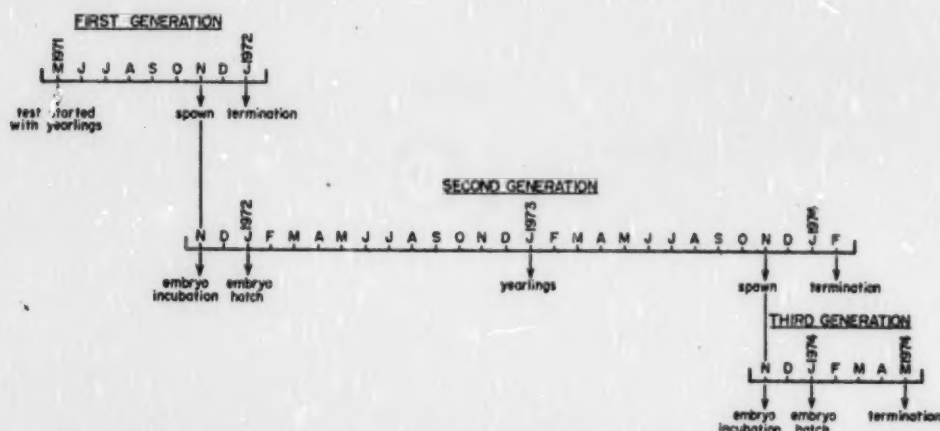


FIGURE 1.—Chronology of three brook trout generations exposed to cadmium.

Cadmium analyses before July 1973 were performed by the extraction of ammonium pyrrolidine dithiocarbamate (APDC)-cadmium complex into methyl isobutyl ketone (MIBK) and subsequent analysis by atomic absorption spectrophotometry (AAS) (Brooks et al. 1967). Duplicate water samples spiked with 2 μg Cd/liter showed that the APDC-MIBK and AAS method gave 99% mean recovery of cadmium for 12 analyses with a 95% confidence interval of ± 4.0 . Differentiation between that cadmium which would pass through a membrane filter of 0.10- μ pore-size (dissolved cadmium) and that cadmium which was present without filtering (acid-soluble cadmium) was made by using the APDC-MIBK and AAS method on 11 unacidified "grab" samples of water from each concentration. The mean percentages of dissolved cadmium and 95% confidence intervals ranged from 71% ± 14.7 to 94% ± 2.9 of the lowest (0.5 μg /liter) and highest (6.4 μg /liter) concentrations of total cadmium, respectively.

After July 1973 anodic film stripping voltammetry (ASV) (Poldoski unpublished report)³ and furnace AAS were used to analyze water samples. These two new methods were faster and more sensitive and agreed well in accuracy with the earlier method. Compari-

sons between the APDC-MIBK and ASV methods, from 9 separate samples at the highest cadmium concentrations, showed that agreement between these two methods was within $\pm 1\%$. The furnace AAS and ASV methods were also compared and found to be within $\pm 7\%$ of 3 samples from the highest cadmium concentration.

Biological

This study involved: (1) exposure of yearling brook trout through spawning (May 1971–February 1972); (2) exposure of the offspring from the initial parental group through spawning (November 1971–February 1974); and (3) exposure of offspring from the second parental group to the early juvenile stage (November 1973–May 1974) (Fig. 1). Biological methods for these three generations will be given separately.

First Generation—Yearlings through Adult Spawning. Yearling brook trout (50–70 g) were obtained from the Cedar Island Hatchery in Brule, Wisconsin. Fish were acclimated to laboratory conditions 4 weeks before starting the exposure. The experiment began during May 1971 when 32 fish per duplicate were placed by stratified random assignment in each of 12 spawning tanks. Sex was determined on all fish during September 1971. Spawners were chosen randomly so that each

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³ Environmental Research Laboratory-Duluth, Duluth, Minnesota 55804.

group had a sex ratio of two males and three females. One spawning substrate was placed in each spawning tank at this time. Spawning commenced in mid-November 1971 and continued until mid-December. Experimental procedures used during spawning and parental termination were the same as those described by McKim and Benoit (1971).

After exposures of 2, 8, 20, and 38 weeks, subsamples of 4–10 fish per duplicate at each concentration were anesthetized with MS-222. Blood was drawn with a syringe by caudal puncture, and gill, liver, kidney, gonad, spleen, and muscle samples were dissected. Tissues were rinsed with distilled water, individually wrapped in cellophane, and frozen along with blood samples for subsequent cadmium analysis (Leonard 1971). The mean percentage recovery and 95% confidence interval of 19 tissue samples spiked with 0.5 and 1.0 μg cadmium was 98 ± 3 .

Second Generation—Embryo through Adult Spawning. Exposure to second-generation trout through one complete life cycle began with the spawning of first-generation fish (Fig. 1). Biological methods used during embryo incubation and alevin-juvenile survival and growth studies were the same as those described by McKim and Benoit (1974). Individual weights were determined from randomly chosen subsamples of 20 juvenile fish per concentration 16 weeks after hatch. Juveniles were transferred from growth chambers to spawning tanks 12 weeks after hatch. Fish exposed to 0.5 μg Cd/liter were terminated 16 weeks after hatch to make space available for another experiment. Sex was determined on all fish during September 1973, and spawners were chosen randomly so that each group had a sex ratio of two males and four females. Two artificial spawning substrates (Benoit 1974) were placed in each tank, and spawning commenced in mid-November 1973. Experimental methods used during spawning and parental termination were the same as those used for first-generation fish.

Ten embryos from the 3.4 μg Cd/liter concentration and the control were preserved in 10% formalin for cadmium-residue analysis. Cadmium residues from alevins preserved in formalin were compared with alevins preserved

by freezing and no significant differences were found between the two methods of preservation. Two to five fish from each duplicate tank were randomly selected at 70, 85, and 105 weeks and were anesthetized in 0.2 g MS-222/liter. Tissues were dissected and analyzed for cadmium residues as previously described for first-generation fish. The remaining fish in each concentration after 105 weeks were finclipped for exposure identification and transferred to control water. After 12 weeks in control water, fish from each former cadmium concentration and controls were anesthetized, and kidney, liver, and gill tissues were dissected and analyzed to determine the loss of accumulated cadmium.

Third Generation—Embryo through 12-Week-Old Juveniles. Third-generation exposures began with the spawning of second-generation fish and continued for 12 weeks after hatch (Fig. 1). Biological methods were the same as those given for the second-generation alevins except that weights were determined on all surviving juvenile fish 12 weeks after hatch.

Composited samples of 10–20 embryos at spawning and four 12-week-old juveniles per duplicate were preserved in 10% formalin for cadmium-residue analysis.

Trout Diets and Treatment of Diseases and Ectoparasites. Fish were fed measured amounts of commercial trout food (Oregon Moist⁴, Glencoe Mills⁵, Silver Cup⁶, and Ewos⁷)⁸ daily. Food rations were calculated bimonthly by multiplying the total body weight of each individual group of exposed fish by a percentage of body weight ranging from 0.7 to 3.4% depending on the average weight of the fish and water temperature.

Diseases and ectoparasites were treated with neomycin, tetracycline, and formalin as described by The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Secondary fungal growth (*Saprolegnia*) was

⁴ Bioproducts Inc., Warrenton, Oregon 97146.

⁵ Glencoe Mills Inc., Glencoe, Minnesota 55336.

⁶ Murray Elevators, Murray, Utah 84107.

⁷ Astra Pharmaceutical Co., Worcester, Massachusetts 01606.

⁸ Selection of these commercial diets was based on pesticide content and availability.

controlled by swabbing the infected area with a 1% solution of malachite green.

Statistics. Growth data were subjected to a one-way analysis of variance with an *F* test ($P = 0.05$) and Student's *t* test ($P = 0.05$) (Steel and Torrie 1960).

Acute Toxicity Tests. Several flow-through acute toxicity tests were conducted at $12 \pm 1^\circ\text{C}$ with 10 yearling (mean 102 g) brook trout per duplicate from the same group used in the first generation chronic test. These tests with 10 fish per duplicate were conducted according to methods described by The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). During the first acute toxicity test (96 h) fish were observed every 24 h, and dead fish were removed with a net. During a second acute exposure (168 h) trout were completely isolated from outside disturbances for 96 h, after which all fish were observed daily for an additional 72 h and dead fish removed as before. Cadmium concentrations measured 12, 22, 44, 90, and 180 $\mu\text{g/l}$ during the first test and 82, 117, 178, 269, and 405 $\mu\text{g/l}$ during the second test.

RESULTS

First Generation

Yearling brook trout exposed to duplicate concentrations of 6.4 $\mu\text{g Cd/liter}$ displayed hyperactivity 8 weeks after the test was initiated. Erratic and uncoordinated swimming movements were followed by loss of equilibrium and paralysis for several minutes. Hyperactivity and periods of paralysis increased with exposure time at this concentration, and all fish eventually died before reaching sexual maturity. Brook trout exposed to 3.4 $\mu\text{g Cd/liter}$ and below did not show hyperactive behavior during the 24-week prespawning period. Males in each duplicate tank at 3.4 $\mu\text{g Cd/liter}$, however, became extremely hyperactive during spawning. When females began their digging and probing activity in the gravel substrate, attending males suddenly began swimming erratically about the tank, lost equilibrium, lapsed into paralysis, and died within several minutes (Table 1). One male, however, managed to spawn twice at 3.4 $\mu\text{g Cd/liter}$ before dying. Females at this

concentration seemed to be less susceptible to cadmium than males, although one female of the six succumbed during the spawning period. The number of mean viable embryos spawned per female was considerably reduced at 3.4 $\mu\text{g Cd/liter}$ because of the effects of cadmium on the males (Table 1). Hyperactivity was not observed at 1.7 $\mu\text{g Cd/liter}$ and below in fish of either sex during the exposure. Growth was not affected significantly during the first-generation exposure. Even though trout were hyperactive in the two highest concentrations, they continued to feed regularly until death or until the test ended. The mean $\pm$ 95% confidence interval of total length and weight of all test fish at thinning were 24.2 ± 1.3 cm and 174 ± 26.6 g, respectively.

After 38 weeks of exposure at 3.4 $\mu\text{g Cd/liter}$, tissue residues were greatest in the kidney, liver, and gill (Fig. 2). Male gonads appeared to accumulate cadmium after 20 weeks; however, as these tissues atrophy and deteriorate after spawning, residues on a per-gram basis increased. Spleen, muscle, and red blood cells accumulated small quantities of cadmium. Bioaccumulations of gill, liver, and kidney tissue were directly related to the exposure concentrations in the test water (Fig. 2).

Residue analyses indicated that an "equilibrium" had been reached during the first 20 weeks. Comparisons of residue based on micrograms of cadmium per gram of tissue and micrograms of cadmium per whole tissue from the kidney, liver, and gill of fish exposed to 3.4 $\mu\text{g Cd/liter}$ showed that even though these tissues had reached a state of equilibrium on a per-gram-of-tissue basis, the total cadmium residue continued to increase as the fish grew.

Second Generation

Hatch and survival of brook trout through the juvenile stage were not affected by cadmium concentrations tested. However, weights of 16-week-old juveniles at 3.4 $\mu\text{g Cd/liter}$ were significantly less than weights of fish exposed to 1.7 $\mu\text{g Cd/liter}$ and below, including controls ($P = 0.05$) (Table 2). An estimated total weight of young produced per female at each concentration was calculated in

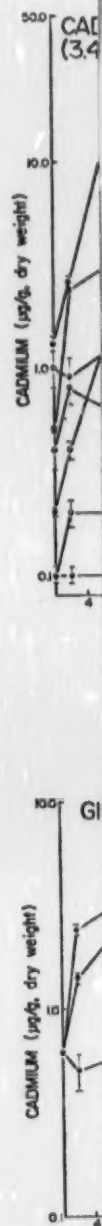


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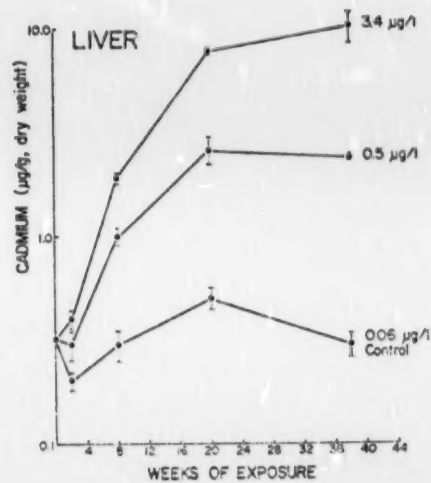
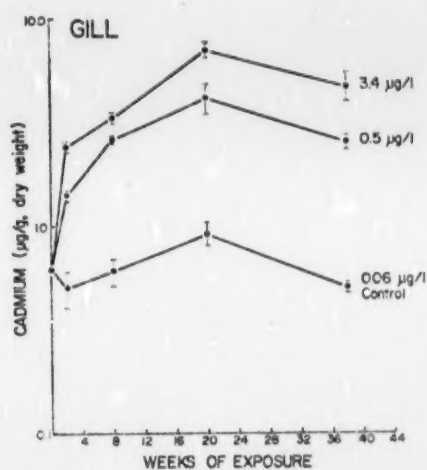
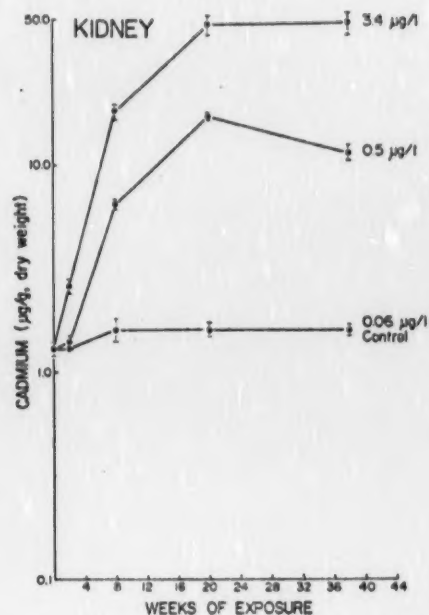
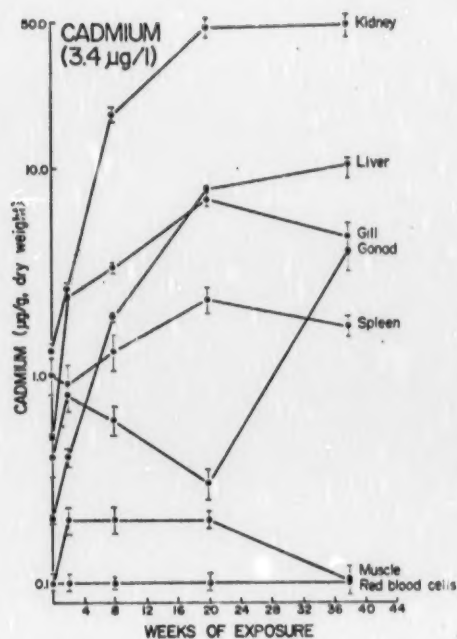


FIGURE 2.—Cadmium residues (µg/g) from tissues of first-generation brook trout exposed to various concentrations of cadmium. Each point represents the mean and standard deviation of 10 samples.

TABLE 2.—Estimated total weight of second- (16-week-old) and third- (12-week-old) generation brook trout produced per female at various cadmium concentrations. Asterisks (*) denote values significantly less than controls (analysis of variance, t test, $P = 0.05$).

Mean total cadmium concentration ($\mu\text{g/liter}$)	Duplicates	1	2	3	4	$1 \times 2 \times 3 \times 4$	Ratio of estimated total weight of young per female (Exposed: Control)
		Estimated ^a embryos/ female	Hatchability (%)	Alevin- juvenile survival (%) ^b	Mean juvenile weight (g)	Estimated total weight of young per female (g)	
Second generation							
3.4	A	98	94 (2) ^d	94	$1.8^* \pm 0.02^* (20)^f$	155	0.05
	B	305	99 (7)	90	$3.81 \pm 0.04 (90)$	1,035	
1.7	A	216	100 (1)	96		700	0.56
	B	508	99 (7)	86	$3.43 \pm 0.08 (20)$	1,479	
0.9	A	401	95 (8)	93		1,190	0.81
	B	216	97 (3)	96	$3.32 \pm 0.13 (20)$	683	
0.5	A	272	99 (5)	96		538	0.46
	B	573	100 (5)	88	$3.31 \pm 0.37 (20)$	1,669	
0.06 (Control)	A	432	100 (5)	94	$3.06 \pm 0.24 (20)$	1,608	1.00
	B						
Third generation							
3.4	A	121	86 (1)	93	$0.58^* \pm 0.03 (35)$	56	0.27
	B	161	93 (4)	72	$1.10 \pm 0.03 (37)$	118	
1.7	A	174	86 (1)	96	$1.16 \pm 0.05 (44)$	166	0.60
	B	87					
0.9	A	194	94 (6)	100	$1.13 \pm 0.04 (47)$	208	1.00
	B	273	71 (6)	98	$0.95 \pm 0.08 (44)$	180	
0.06 (Control)	A	200	87 (2)	98	$1.38 \pm 0.09 (40)$	230	1.00
	B						

^a Number of viable embryos produced per spawning female $\times$ the probability of spawning (i.e., total spawners/total females = 0.7 for second- and 0.5 for third-generation).

^b Fifty fish exposed in each duplicate.

^c All males died either during or shortly after spawning.

^d Number of spawnings checked for hatchability.

^e Mean and 95% confidence interval.

^f Number of fish sampled.

^g Disease-related embryo death.

Table 2 by multiplying the number of embryos per female $\times$ percent hatch $\times$ percent survival $\times$ mean weight. The combined reduction in number of viable embryos per female and retarded growth of offspring at 3.4 $\mu\text{g Cd/liter}$ drastically reduced the estimated total weight of 16-week-old young produced per female. Ratios (estimated weight of young produced from exposed fish)/(estimated weight of young produced from control fish) showed a marked decrease (95%) at 3.4 $\mu\text{g Cd/liter}$, whereas at concentrations of 1.7 $\mu\text{g Cd/liter}$ and below ratios approached 50% of controls or greater (Table 2). Survival of offspring from hatch to sexual maturation was normal, and hyperactivity was not observed throughout this period (88 weeks). During spawning, however, males at 3.4 $\mu\text{g Cd/liter}$ became hyperactive and died as did first-generation males at this concentration. One male was able to spawn once at 3.4 $\mu\text{g Cd/liter}$

before dying (Table 1). Hyperactivity was never observed at 1.7 $\mu\text{g Cd/liter}$ and below, and spawning appeared normal.

Mean residues in 10 embryos spawned in 3.4 $\mu\text{g Cd/liter}$ differed little from residues in control embryos (0.13 and 0.10 $\mu\text{g Cd/g}$, respectively). Mean residues ($\mu\text{g Cd/g}$) and standard deviations from five trout after 70 weeks of exposure to 3.4 $\mu\text{g Cd/liter}$ were, respectively: kidney, 64.8 ± 21.6 ; liver, 9.0 ± 2.0 ; gill, 5.1 ± 2.6 ; spleen, 6.0 ± 6.2 ; gonad, 2.6 ± 1.8 ; muscle tissue, 0.05 ± 0.02 . These values compared closely with those measured in first-generation fish after 38 weeks of exposure to the same concentration (Fig. 2). Bioaccumulation measured in the kidney, liver, and gill of second-generation fish after 70 weeks of exposure to 3.4 $\mu\text{g Cd/liter}$ showed that sometime before the first sampling these tissues had reached a state of "equilibrium" (based on micrograms of cad-



FIGURE 3.—and second-generation brook trout for first-generation samples.

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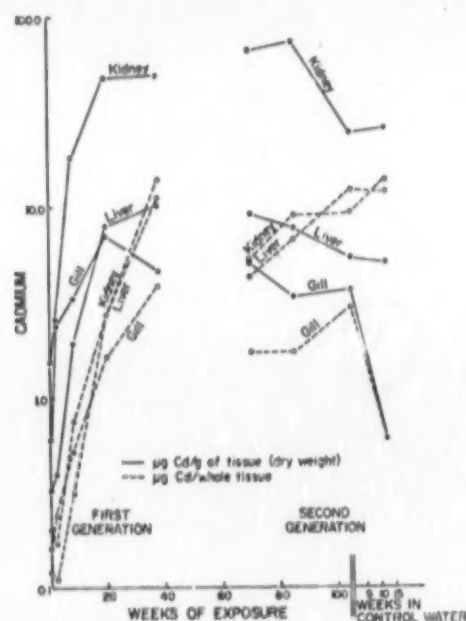


FIGURE 3.—Cadmium residues from tissues of first- and second-generation brook trout exposed to 3.4 $\mu\text{g Cd/liter}$; and loss from tissues of second-generation trout placed in control water. Each point for first-generation fish represents the mean of 10 samples; for second-generation fish, five samples.

mium per gram of tissue) at levels remarkably close to residues measured in first-generation fish after 20 weeks of exposure (Fig. 3). Comparisons of second-generation kidney, liver, and gill residues based on micrograms of cadmium per gram of tissue and micrograms of cadmium per whole tissue also showed the same relationship to tissue growth as was described for first-generation fish.

When fish that had been exposed to 3.4 $\mu\text{g Cd/liter}$ for 105 weeks were placed in control water for 12 weeks, loss of total cadmium residue from gill tissue was 79% on a whole-tissue basis and 83% on a per-gram-of-tissue basis (Fig. 3). Gill values approached those measured in control fish ($0.2 \pm 0.06 \mu\text{g Cd/g}$) and indicate a rapid loss of cadmium from this tissue. Residues remained about the same in the liver of these fish, however, and increased slightly in the kidney. The cadmium that continued to accumulate in the

kidneys was probably translocated from other parts of the body. Loss from the gill, liver, and kidney of trout previously exposed to 0.9 and 1.7 $\mu\text{g Cd/liter}$ followed a pattern similar to that described for fish exposed to 3.4 $\mu\text{g Cd/liter}$.

Third Generation

The exposure of fish embryos through 12 weeks after hatch produced results similar to those described for second-generation juveniles. Hatching and survival of larvae were not affected by cadmium, but 12-week-old juveniles exposed at 3.4 $\mu\text{g Cd/liter}$ weighed significantly less than fish exposed to 1.7 $\mu\text{g Cd/liter}$ and below ($P = 0.05$) (Table 2). Estimated total weights of 12-week-old young produced per female at 3.4 $\mu\text{g Cd/liter}$ was much lower than weights produced at 1.7 $\mu\text{g Cd/liter}$ and below. Ratios of weights from exposed and control fish (as defined for second-generation fish) also showed a marked decrease (73%) at 3.4 $\mu\text{g Cd/liter}$. Abnormal behavior was not observed throughout the 12-week exposure period.

Residues from samples of 10-20 embryos spawned in 0.06 (control), 0.9, 1.7, and 3.4 $\mu\text{g Cd/liter}$ were 0.12, 0.11, 0.12, and 0.21 $\mu\text{g Cd/g}$, respectively. Whole-body residues of four 12-week-old juveniles exposed to 0.06 (control), 0.9, 1.7, and 3.4 $\mu\text{g Cd/liter}$ were 0.11, 0.68, 0.65, and 1.26 $\mu\text{g Cd/g}$, respectively.

MATC

Results of the present exposure involving three generations of brook trout indicate that the MATC for brook trout in Lake Superior water with a hardness of 44 mg/liter (as CaCO_3) and a pH range of 7 to 8 lies between 1.7 and 3.4 $\mu\text{g Cd/liter}$. This calculation was based upon effects of 3.4 $\mu\text{g Cd/liter}$ on survival of first- and second-generation adult males during spawning and on growth of second- and third-generation juveniles.

Acute Toxicity

The first 96-h acute toxicity test (12-180 $\mu\text{g Cd/liter}$), in which fish were observed daily, produced an essentially flat mortality-concentration curve (i.e., 95% mortality at 12

and 180 $\mu\text{g Cd/liter}$). Survival was 100% after 96 h in all tanks during the second acute toxicity test (82–405 $\mu\text{g Cd/liter}$) in which fish were not observed daily. Results, however, during the remaining 72 h of the second toxicity test, in which fish were observed daily, produced another flat mortality-concentration curve (i.e., 80% mortality at 82 and 405 $\mu\text{g Cd/liter}$). Attempts, therefore, to determine a 96-h LC50 and an application factor were unsuccessful. Acute hyperactivity was similar to that described previously for chronically exposed fish.

DISCUSSION

The toxic mode of action of cadmium on the nervous system of first- and second-generation brook trout males is not unique. Flagfish (*Jorandella floridae*) exposed to 16 $\mu\text{g Cd/liter}$, as recently reported by Spehar (1974), showed similar hyperactivity; males were more distressed during spawning activity than females. Quentin H. Pickering (Newtown Fish Toxicology Station, Cincinnati, Ohio) has observed signs of nervous disorders in fathead minnows chronically exposed to 110 $\mu\text{g Cd/liter}$, and males are apparently slightly more sensitive than females. Eaton (1974) found that bluegills exposed to 80 $\mu\text{g Cd/liter}$ became increasingly subject to convulsions, which sometimes resulted in death. Male bluegills, however, did not appear to be more sensitive than females. Largemouth bass (*Micropterus salmoides*) and bluegills exposed by Cearley and Coleman (1974) to 8 and 85 $\mu\text{g Cd/liter}$, respectively, also exhibited hyperactivity, but a difference in sensitivity between sexes was not mentioned. These authors theorized that the toxic manifestations in the nervous system could be consistent with the inhibition of the enzyme acetylcholinesterase, resulting in death by paralysis of the respiratory center. Intraperitoneal injections and doses of cadmium in the drinking water of rats and mice produced hyperactivity and contributed to a progressive mortality of males, but less so of females (Schroeder 1964, 1965; Schroeder et al. 1966). Schroeder (1965) also suggested a possible correlation between human hypertensive diseases and residues of cadmium found in the kidney and urine.

These findings seem to suggest that cadmium's toxic mode of action may be quite similar in most animals.

Our study demonstrated that the "no-effect" concentration was constant with succeeding generations. This result agrees with data from McKim and Benoit (1974), Holcombe et al. (1976), and McKim et al. (1976), who showed that the no-effect concentration was constant for several successive generations of brook trout exposed to copper, lead, and mercury, respectively. These life-cycle exposures strongly indicate the validity and usefulness of conducting shorter partial-life-cycle bioassays for determining a MATC.

The effect of cadmium on the early life of brook trout described in this report is similar to that reported for fathead minnow embryos (Pickering and Gast 1972) and bluegill larvae (Eaton 1974). The chronic effect concentrations reported for these species and the brook trout were equal to or less than those causing adverse effects during other life stages of these fish. Thus cadmium MATC values could be established for fish through the testing of early life stages in place of costly long-term life-cycle experiments.

Since residues in the kidney, liver, and gill of laboratory-exposed brook trout apparently reach states of equilibrium, analysis of these tissues in wild trout populations might be useful in determining whether they have been or are currently being subjected to sublethal concentrations of biologically active cadmium regardless of how much total cadmium was measured in the water at the time of sampling. Residues measured in wild trout approximating those previously mentioned in laboratory fish exposed to 3.4 $\mu\text{g Cd/liter}$ could be detrimental or could indicate detrimental exposures, since larval growth and adult survival were decreased at this and higher exposure concentrations. Similar cadmium residue distributions and equilibria have recently been reported in the kidney, liver, and gill of rainbow trout (*Salmo gairdneri*) and dace (*Triborodon halconensis*) by Kumada et al. (1973); in largemouth bass and bluegill by Cearley and Coleman (1974); in the bluegill by Eaton (1974); and in the white catfish (*Ictalurus catus*) by Rowe and Massaro

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(1974). Kidney and liver tissues were also shown by Schroeder et al. (1965) to be the primary sites of cadmium deposition in rats and mice after chronic exposures via drinking water and diet.

Brook trout were able to spawn successfully at 3.4 μg Cd/liter and below. This finding tends to agree with the reports of Sangalang and O'Halloran (1972), who found extensive haemorrhagic necrosis in the testes of male brook trout exposed to 10 and 25 μg Cd/liter, but were unable to find histological changes at 1 and 2 μg Cd/liter.

Our data on cadmium loss from gill, kidney, and liver of brook trout agree closely with those of Kumada et al. (1973). They found that when rainbow trout were exposed to cadmium for 30 weeks and were then placed in clean water for 10 weeks cadmium concentrations in the gill decreased but those in the kidney and liver remained unchanged.

Results of the acute toxicity tests were apparently related to outside disturbances and resultant hyperactivity and interactions between fish at all concentrations tested. Acute effects of cadmium on brook trout have recently been tested by Robert A. Drummond (Environmental Research Laboratory-Duluth, Duluth, Minnesota). He isolated and exposed 28 yearling trout individually to 40 μg Cd/liter in Lake Superior water; after 144 h only 14% of the fish had died. Similar inconsistencies and problems associated with the acute toxicity of cadmium have also been reported for fathead minnows (Pickering and Gast 1972), rainbow trout (Ball 1967), and rainbow trout and dace (Kumada et al. 1973).

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